Plant Omics Journal POJ

POJ 7(6):510-516 (2014) ISSN:1836-3644

Research Note

# Variation in bioactive principles of Korean black raspberry (*Rubus coreanus* Miquel) during ripening

**Tae Kyung Hyun[1], Yeonggil Rim[1], Ekyune Kim[2], Ju-Sung Kim*[3,4]**

**[1]Division of Applied Life Science, Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, Republic of Korea**
**[2]College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Republic of Korea**
**[3]Majors in Plant Resource and Environment, College of Agriculture & Life Sciences, SARI, Jeju National University, Jeju 690-756, Korea**
**[4]The Research Institute for Subtropical Agriculture and Biotechnology, Jeju National University, Jeju 690-756, Korea**

***Corresponding author: aha2011@jejunu.ac.kr**

**Abstract**

The present investigation was carried out to appraise the variation in antioxidant and antidiabetic potential of Korean black raspberry during its ripening process. Bioactive principles of Korean black raspberry were evaluated by in vitro antioxidant and antidiabetic assays including free radical scavenging activity, reducing power by $Fe^{3+}$-$Fe^{2+}$ transformation and α-glucosidase inhibitory activity. Overall, an extract of unripe fruit contained the highest levels of total phenolic and flavonoid contents, radical scavenging activity, reducing power, and α-glucosidase inhibitory activity, compared with extracts of fruit at other stages of ripening. On the other hand, total anthocyanin content increased with the progression of fruit ripening, indicating that the metabolic shift from proanthocyanidins to anthocyanins in the flavonoid biosynthesis pathways influenced the antioxidant and antidiabetic activities of Korean black raspberry. These results suggest that the different stages of maturation have profound effects on the secondary metabolites and variations in bioactive principles of Korean black raspberry. In addition, based on *in-silico* gene expression analysis, we found that catechin-induced apoptosis in human breast cancer cell line MCF-7 is mediated by the induction of death ligand-mediated extrinsic apoptosis pathways.

**Keyword:** Ripening, antioxidant activity, α-glucosidase inhibitory effect, catechin, *Rubus coreanus* Miquel.
**Abbreviations:** TPC_Total phenolic content; TFC_Total flavonoid content; TAC_Total anthocyanin content.

## Introduction

During the last few decades, the great interest in traditional plants and their medicinal value has promoted the consumption of fruits, vegetables, and herbs for improving personal health. The medicinal properties of these plants are due to their ethnopharmacological activities, low toxicity, and economic viability (Chew et al., 2012). Several phytochemicals, such as khellin from *Ammi visnage* (L) Lamk, galegine from *Galega officinalis* L., papaverine from *Papaver somniferum* L., curcumin from *Curcuma longa* Linn., and epigallocatechin-3-gallate from green tea, have been identified and showed promising pharmaceutical activities including antidiabetic activity, anticancer activity and anti-inflammatory activity (Cragg and Newman, 2013). In addition, these compounds have been used as important resources in the synthesis of drugs that are artificially modified versions of phytochemicals (Cragg and Newman, 2013). These findings indicate that traditional plants are useful not only as supplementary health foods but also a primary source for drug development. The Korean black raspberry (*Rubus coreanus* Miquel) is a perennial wild-type berry, which belongs to the *Rosaceae* family (Park et al., 2012). Since it has been used as a folk medicine, current pharmaceutical studies have further revealed its pharmaceutical applications based on its antioxidant, antibiotic, anti-inflammatory, antiviral, and anticancer activities (Kim et al., 2001; Wang and Stoner, 2008; Lim et al., 2012). On the basis of phytochemical analyses, it has been suggested that the pharmaceutical activities of Korean black raspberry are related to its phytochemical constituents, including anthocyanins, tannins, catechins, and teriterpenoids (Yoon et al., 2003; Kim et al., 2011). Although the ripe fruits have usually been used as a source for investigating the pharmaceutical activities of Korean black raspberry, the dried unripe fruits, commonly known as Bokbunja in Korea, have been used for centuries as a traditional herbal medicine (Shin et al., 2002). This indicates that the unripe fruits of Korean black raspberry may be a better source for investigating its pharmaceutical value than the ripe fruits. Therefore, in this study, we investigated the variation in bioactivities of Korean black raspberry during its ripening process. In addition, based on *in-silico* gene expression analysis, we analyzed the possible action of catechin, which is known to be one of the active compounds from Korean black raspberry, on cells of the human breast cancer cell line MCF-7.

## Results and Discussion

### *Variation in total phenolic, flavonoid, and anthocyanin contents at the three ripening stages of Korean black raspberry*

It is generally accepted that different parameters, including variety, season, climatic conditions, and stages of maturity influence the phytochemical compositions of plant materials (Cordenunsi et al., 2002). Therefore, in order to determine the variability in bioactive principles during the fruit ripening process, we initially analysed the changes in total phenolic, flavonoid, and anthocyanin contents (TPC, TFC, and TAC, respectively) during the three ripening stages of fruits. The results of the distribution of TPC, TFC, and TAC in relation to fruit maturity/ripening stage are presented in Table 1. The concentration of phenolics and flavonoids were generally higher in the extract from unripe samples compared to the semi-ripe and fully ripe samples, whereas there was an accumulation of anthocyanins as ripening progressed. During the ripening process, the amount of proanthocyanidins, classed as flavonoids, has been found to decrease in many berry species, whereas anthocyanins accumulate in their fruits (Zhang et al., 2011; Lee et al., 2012). Proanthocyanidins are essential for the astringency and bitterness of fruits and they exhibit a broad range of pharmacological properties (Chen et al., 2012). Therefore, the increasing level of total anthocyanins during the ripening process of Korean black raspberry indicates that the switch in flavonoid biosynthesis from proanthocyanidins to anthocyanins might reflect the changing strategy from protecting fruit to promoting fruit consumption by fruit-eating animals.

### *Influence of fruit ripening on the antioxidant activity*

Antioxidants are molecules that inhibit the oxidation of other molecules, and thereby protect organisms against the damage caused by reactive oxygen species that contain a number of unpaired electrons. The oxidative damage of cellular components, including lipids, proteins, and nucleic acids are involved in various diseases such as cancer, diabetes, and neurological diseases (Surveswaran et al., 2007). Because they can be used safely over the long-term, natural antioxidants have begun to gain worldwide interest in healthcare promotion (Kim et al., 2010). The vitamins, glutathione, carotenoids, anthocyanins, and phenolics present in fruits are effective scavengers of free radicals, and changes in these compounds during the ripening process is correlated with the antioxidant activity of fruits (Gull et al., 2012). In the case of Korean black raspberry, extracts of the unripe fruits displayed the highest antioxidant activity ($RC_{50}$ = 153.4±5.7 μg/ml) compared with the extracts of fruit at other stages of ripening (Fig. 1B). Anthocyanins have a wide range of health benefits mediated by their antioxidant activities. Although the extract of fully ripe fruits contained the highest level of anthocyanins (580 ± 40 μg/g), this extract exhibited the lowest free radical scavenging activity ($RC_{50}$ = 689.0 ± 15.6 μg/ml). To further characterize the antioxidant properties in the extracts, the reducing power of Korean black raspberry extracts was analyzed. As shown in Fig. 2, the reducing potential of each extract was found to increase in a dose-dependent manner. As expected, the unripe fruits exhibited the highest reducing potential, followed by the semi-ripe fruits and fully ripe fruits. On the basis of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity analysis, the extract of unripe fruits showed higher radical scavenging activity than butylated hydroxytoluene (BHT) (Fig. 1B). However, this extract contained lower reducing power than BHT (Fig. 2). This indicates that the antioxidant activity of Korean black raspberry extracts is due their reaction with free radicals, thereby converting them into more stable products. In addition, the decreasing level of antioxidant activities during the ripening process is mainly mediated by TPC and TFC, but not by TAC.

### *Influence of fruit ripening on the α-glucosidase inhibitory activity*

α-Glucosidase is an enzyme involved in the intestinal absorption of starch by catalyzing the conversion of starch to glucose. The inhibition of this enzyme would result in the reduced uptake of dietary carbohydrates and suppression of postprandial hyperglycemia (Hsieh et al., 2010). Since natural products such as flavonoids, alkaloids, anthocyanins, glycosides, and phenolic compounds have been characterized as potential α-glucosidase inhibitors (Kumar et al., 2011), the exploration of medicinal plant products is an important issue in the development of antidiabetic agents. To investigate the antidiabetic potential of Korean black raspberry extracts and the variation in antidiabetic activity during the ripening process, we analyzed the effect of each extract on α-glucosidase activity. As shown in Fig. 3, the extract from unripe fruits showed the inhibitory activity against α-glucosidase, with an $IC_{50}$ value of 2.02±0.02 μg/ml, whereas the lowest inhibitory effect was observed from the extract of fully ripe fruits ($IC_{50}$ value of 8.6±0.09 μg/ml). These findings, together with antioxidant activities, indicate that the fruit ripening stage has an effect on bioactivities of Korean black raspberry. On the basis of phytochemical analyses, triterpenoids, tannins, diterpenes, and catechins have been isolated from the unripe fruits of Korean black raspberry (Do et al., 1988; Seo et al., 2011). Catechin is the most abundant polyphenolic compounds in plant materials, and the amount of catechin decreases during the ripening process of fruits, including Korean black raspberry (Taniguchi et al., 2007; Kim et al., 2011; Agudelo-Romero et al., 2013). In addition, our previous study has been shown the decreasing amount of catechin during the ripening process of Korean black raspberry (Hyun et al. 2014). Furthermore, catechin is known as potential inhibitors of α-glucosidase and antioxidant agent (Pedrielli et al., 2001; Xu et al., 2013). These observations indicate that catechin is one of the potential active compounds in Korean black raspberry extract.

### *Possible mechanism for the apoptosis-promoting action of catechin*

Catechin is natural compound that belongs to the class of flavan-3-ols, and has exhibited cytostatic properties in many tumor models (Alshatwi, 2010). (−)-epigallocatechin-3-gallate (EGCG), which is major catechin in green tea, affects a range of signaling and metabolic pathways that may result in the inhibition of cancer cell growth and induction of cancer cell apoptosis (Yu et al., 2014). Catechin-induced apoptosis is based on the ability of catechin to increase the expression of pro-apoptotic genes, *caspase-3*, *-8* and *-9*, which are situated at pivotal junctions in apoptosis pathways (Alshatwi, 2010). This indicates that the anticancer activity of Korean black raspberry extract mediated by its ability to activate the caspase pathway (Kim et al., 2005) might be due to the presence of catechin, suggesting the importance of catechin as an active compound in Korean black raspberry. The possible function of catechin in the apoptosis pathway has been well addressed (Sutherland et al., 2006; Park et al., 2009). However, so far, the early events induced by catechin in the apoptosis pathway are not fully understood. Therefore, to investigate the action of catechin in

**Table 1.** Variation of total phenolic, total flavonoids and total anthocyanin contents during the ripening process of Korean black raspberry.

| Stages | Total phenol (mg CAE/g)[1)] | Total flavonoid (mg QAE/g)[2)] | Total anthocyanins (μg/g)[3)] |
|---|---|---|---|
| Unripe | 372.7±6.6a[4)] | 7.5±0.3a | 80±1c |
| Semi-ripe | 140.3±6.3b | 2.9±0.3c | 230±20b |
| Fully ripe | 123.5±8.2c | 3.9±0.3b | 580±40a |

[1)] Total phenolic content analyzed as catechin equivalent (CAE) mg/g of extract, values are the average of triplicates.
[2)] Total flavonoid content analyzed as quercetin equivalent (QE) mg/g of extract, values are the average of triplicates.
[3)] Total anthocyanins content analyzed as cyanidin 3-glucoside equivalent μg/g of extract, values are the average of triplicates.
[4)] Each value represents the mean±SD, and the means were significantly different as calculated from a paired Duncan's test at $p < 0.05$.

cancer cells, systematic analyses of microarray data were carried out. When MCF-7 cells were treated with catechin for 6 h, a total of 7,997 genes ($\log_2$ ratio ≥ 0.5 and ≤ -0.5) were differentially expressed between catechin treated samples and DMSO-treated samples; these included both up-regulated (5,869 genes) and down-regulated genes (2,128 genes). The top 500 up-regulated and down-regulated genes were grouped into functional categories according to the Gene Ontology (GO) terms using the online program PANTHER. As shown in Fig. 4, GO analysis of the regulated genes revealed various affected biological processes, mainly cellular process, metabolic process, cell communication, developmental process, immune system process.

To analyze the responses of the apoptosis-linked genes, all differentially expressed genes ($\log_2$ ratio ≥ 0.5 and ≤ -0.5) were inserted into the apoptosis pathway (death ligand-mediated extrinsic apoptosis pathway) in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. In the death ligand-mediated extrinsic apoptosis pathway, intracellular protein complexes, including death ligands such as tumor necrosis factor (TNF, formerly known as TNFα), TNF-related apoptosis-inducing ligand (TRAIL), interleukin 1 (IL-1), and Fas ligand (Fas-L), and death receptors such as TRAIL-receptor (TRAIL-R), TNF-receptor, Fas, and IL-1 receptor, activate caspase-8 and caspase-10, which, in turn, leads to the activation of effector caspase-3, -6, and -7 (Sakamaki and Satou, 2009; Fecker et al., 2010). When MCF-7 cells were treated with catechin, *TNFα*, *TRAIL*, and *IL-1* were up-regulated, whereas the expression of *Fas-L* was reduced (Fig. 5). TRAIL induces apoptosis by signaling via the death domain containing TRAIL receptors (TRAIL-Rs) (Pan et al., 1997). It is known that TRAIL induces apoptosis of human tumor cells, but not normal cells (Gura, 1997), indicating that the systemic administration of catechin might be useful for the treatment of cancer. Receptor-interacting protein 1 (RIP1, also known as RIPK1) acts as a key signal transducer involved in apoptosis and necroptosis (Christofferson et al., 2012). When apoptosis is blocked, necroptosis is induced by the kinase activity of RIP1 as a caspase-independent pathway of necrotic cell death (Christofferson et al., 2012). As shown in Fig. 5, catechin induced the expression of *RIP1*, which is required to mediate the assembly of the RIP1/FADD (Fas-associated death domain)/caspase-8 complex (Basit et al., 2012). Intriguingly, the expression level of *baculoviral IAP repeat-containing protein 7* (*BIRC7*), which is one of the IAPs (inhibitor of apoptosis proteins), increased after treatment with catechin (Fig. 5). Similarly, it has been shown that curcumin induces the expression of *BIRC7* (8 h after treatment). However, the expression of *BIRC7* was reduced and the growth of HA22T/VGH cells was inhibited by long-term treatment with curcumin (Notarbartolo et al., 2005), indicating the transient induction of *BIRC7* by curcumin and also by catechin. Taken together, these findings indicate that catechin might play the role of a signal molecule that mediates activation of the death ligand-mediated extrinsic apoptosis pathways.

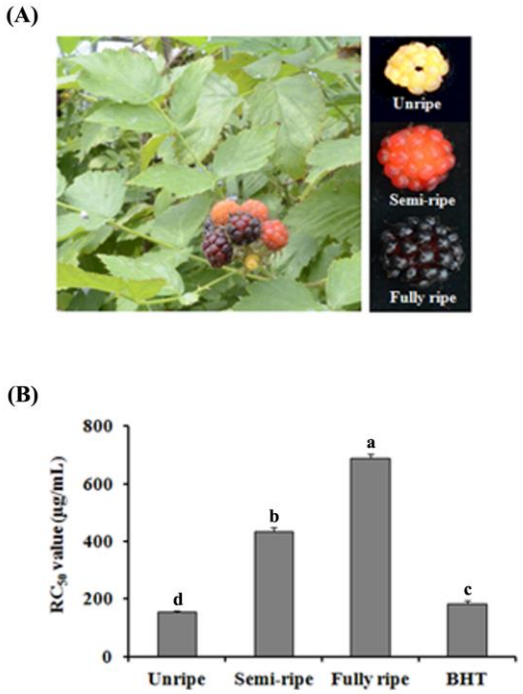


**Fig 1.** Antioxidant activities of Korean black raspberry extracts. (A) General view of *Rubus coreanus* Miquel, (B) DPPH-radical scavenging activity of Korean black raspberry extracts. Bar graph showing $RC_{50}$ values for each extract from the DPPH assay. The $RC_{50}$ values represent the concentration of extract required to reduce the absorbance of the DPPH radical by half. Means values with different superscripts letters were significantly different at $p < 0.05$.

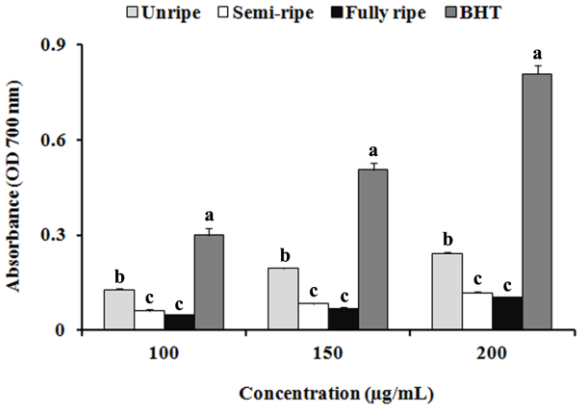


**Fig 2.** Reducing power activity of Korean black raspberry extracts. Values are the average of triplicate experiments and are represented as the mean ± standard deviation. Means values with different superscripts letters were significantly different at $p < 0.05$.

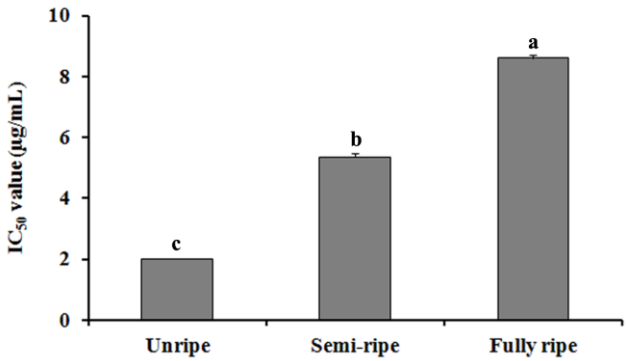


**Fig 3.** Inhibitory effect of Korean black raspberry extracts on α-glucosidase activity. Bar graph showing $IC_{50}$ values for each extract from the α-glucosidase inhibitory assay. Means values with different superscripts letters were significantly different at $p < 0.05$.

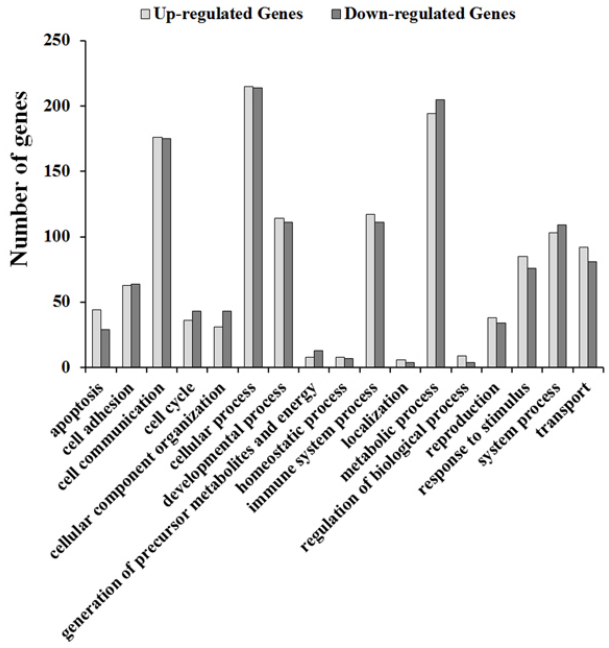


**Fig 4.** Functional categories of genes regulated in MCF-7 cells after 6 h treatment with catechin. *In-silico* gene expression analysis was carried out using microarray dataset for catechin-treated MCF-7 cells. Top 500 up- and down-regulated genes were classified into biological processes including 17 sub-groups. Each bar represents the number of genes that were up- or down-regulated in the respective groups.

## Materials and Methods

### *Plant materials and extraction*

Wild-type Korean black raspberries (*Rubus coreanus* Miquel) were grown in the Gochang Black Raspberry Research Institute, the Republic of Korea. Fruit samples (50 fruits of the same maturation degree collected from 5 to 10 individual plants) were collected during the three fruit ripening stages: orange-turning fruit [15 days post-anthesis (15 DPA), unripe stage], red fruit (20 DPA, semi-ripe stage), and dark red fruit (25 DPA, fruits attached to the crown, fully ripe stage) as shown in Fig. 1A. After harvest, samples were dried at 60°C for 2 days, followed by grinding of samples.

The ground materials were soaked in 70% ethanol for 2 h, placed in an ultrasonic bath and sonicated at 55°C. After filtration, each extract was evaporated using a rotary vacuum evaporator.

### *Analysis of total phenolic, flavonoid, and anthocyanin contents*

The total phenolic content was determined using Folin-Ciocalteu reagent, with catechin as a standard phenolic compound. Each extract (0.1 ml) was treated with 50 µl of 2 N Folin-Ciocalteu reagent for 3 min at room temperature, followed by the addition of 0.3 ml of 20% sodium carbonate and incubation for 2 h at room temperature. After mixing with 1 ml of distilled water, absorbance was measured at 725 nm using a UV-spectrophotometer. The results were expressed as milligrams of catechin equivalents (CAE) per gram of extract using the equation obtained from the standard catechin graph.

To analyze the total flavonoid content in each extract, 0.5 ml of each extract was added to test tubes containing 0.1 ml of 10% aluminum nitrate (w/v), 0.1 ml of 1 M potassium acetate, and 4.3 ml of 80% ethanol. After 40 min incubation at room temperature, absorbance was determined at 415 nm. The total flavonoid content was determined as milligrams of quercetin equivalents (QE) per gram of extract.

The total anthocyanin content was determined using the pH differential method described by Chen et al. (2012). Absorbance was measured at 510 and 700 nm in buffers at pH 1.0 and 4.5. Anthocyanin content was calculated as cyanidin 3-glucoside with an extinction coefficient of 26900 L $cm^{-1}$ $mg^{-1}$ and molecular weight of 449.2. The results were expressed as milligrams of cyanidin 3-glucoside equivalents per gram of extract.

### *DPPH radical-scavenging activity*

Radical scavenging activity was measured using DPPH according to the method described by Hyun et al. (2013). Each extract was redissolved in 70% ethanol (EtOH). Twenty microliter extracts at different concentrations was mixed with 3.98 ml methanol and 1 ml DPPH (0.15 mM in methanol). After incubation at room temperature for 30 min, DPPH radical inhibition was measured at 517 nm using a spectrophotometer. The $RC_{50}$ (50% reduction of DPPH radicals) value of each sample was calculated from a graph of radical scavenging activity versus extract concentration. BHT was used as the standard.

### *Measurement of reducing power*

Reducing power assay is based on the reduction of colourless ferric complex ($Fe^{3+}$ tripyridyltriazine) to blue-colored ferrous complex ($Fe^{2+}$ tripyridyltriazine) by the action of electron donating antioxidants at low pH (Irshad et al., 2012). This assay has been used for measuring a wide concentration range of antioxidant activities and capacities (Hyun et al., 2013). The reducing power of each extract was determined according to the method described by Hyun et al. (2013). Serial dilutions (200, 150, and 100 µg/ml) of the extract were prepared in 0.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, mixed with 2.5 ml of 10% trichloroacetic acid, and then centrifuged at 6,500 rpm for 10 min. The supernatant was mixed with distilled water containing ferric chloride. The absorbance of this mixture was measured at 700 nm using a UV-visible spectrophotometer.

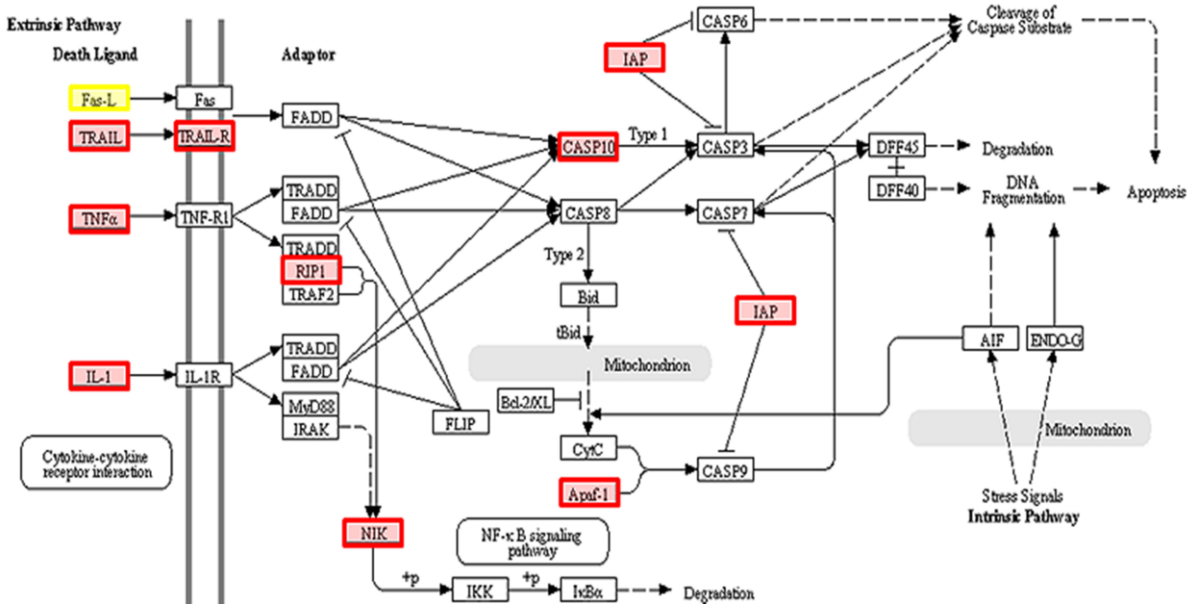


**Fig 5.** KEGG analysis of the extrinsic apoptosis pathway. The differentially expressed genes ($\log_2$ ratio ≥ 0.5 and ≤ -0.5) were inserted into the corresponding gene in the extrinsic apoptosis pathway (hsa04210) diagram referenced from the KEGG pathway database. The red and yellow boxes indicate the up- and down-regulated genes, respectively, in catechin-treated MCF-7 cells. According to the KEGG notation, solid arrows represent molecular interaction or relationship and dashed arrows represent indirect effect.

***Assay for α-glucosidase inhibitory activity***

α-Glucosidase inhibitory activity was determined according to the method described by Hyun et al. (2014) with minor modifications. Fifty microliter α-glucosidase (0.5 U/ml) was pre-incubated with 50 μl of each extract and 50 μl of 0.2 M potassium phosphate buffer (pH 6.8) at 37°C for 15 min, and then the enzyme reaction was started by adding 3 mM *p*-nitrophenyl glucopyranoside (pNPG). The enzymatic reaction was allowed to proceed at 37°C for 10 min, and was then stopped by adding 750 μl of 0.1 M $Na_2CO_3$. The α-glucosidase activity was analyzed spectrophotometrically at 405 nm by measuring the quantity of *p*-nitrophenol release from pNPG. The α-glucosidase inhibitory effects of each extract were calculated as a percentage of the control using the following formula:

Inhibition rate (%) = $[1-(Abs_{sample} - Abs_{blank}) / Abs_{control}] \times 100$,

where $Abs_{sample}$ represents the absorbance of the experimental sample, $Abs_{blank}$ represents the absorbance of the blank, and $Abs_{control}$ represents the absorbance of the control.

***In-silico gene expression analysis***

The microarray data for catechin-treated MCF-7 cells (a human breast adenocarcinoma cell line) was available in the NCBI Gene Expression Omnibus (GEO) database under the series accession number GSM119261 (MCF-7 cells treated with catechin for 6 h) and GSM119262 (MCF-7 cells treated with DMSO for 6 h). Probe sets corresponding to genes were identified using an online program R (http://www.r-project.org/). The expression levels for each gene in the catechin-treated sample were calculated relative to its expression in the DMSO-treated sample.

For the functional annotation, the top 500 up-regulated and down-regulated genes were searched against the Gene Ontology (GO) database using the online program PANTHER (http://www.pantherdb.org/).

For apoptosis pathway enrichment analysis, we mapped all differentially expressed genes ($\log_2$ ratio ≥ 0.5 and ≤ -0.5) with the terms in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/kegg).

***Statistical analysis***

All the experiments were conducted for three independent replicates, and data were expressed as mean±standard deviation. Statistical analyses were performed by ANOVA and Duncan's test was used to determine the significance of differences between the groups. Differences at $p < 0.05$ were considered significant.

**Conclusion**

In this study, we analyzed the variation in antioxidant and antidiabetic activities during three ripening stages of Korean black raspberry. During the ripening process, antioxidant and antidiabetic activities mediated by the TPC and TFC of fruits were reduced, indicating that the fruit ripening stages have profound effects on the pharmaceutical value of Korean black raspberry. In addition, the analysis of the microarray data revealed the effects of catechin on the death ligand-mediated extrinsic apoptosis pathways in MCF-7 cells, suggesting that catechin-induced apoptosis is initiated by the induction of death ligands. Nonetheless, further investigations, including functional assays and *in vivo* models, are needed to analyze the pharmaceutical value of Korean black raspberry.

**Acknowledgements**

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2014R1A1A2004432), Republic of Korea.

**References**


Agudelo-Romero P, Erban A, Sousa L, Pais MS, Kopka J, Fortes AM (2013) Search for transcriptional and metabolic markers of grape pre-ripening and ripening and insights into specific aroma development in three Portuguese cultivars. PLoS One. 8:e60422.

Alshatwi AA (2010) Catechin hydrate suppresses MCF-7 proliferation through TP53/Caspase-mediated apoptosis. J Exp Clin Cancer Res. 29:167.

Basit F, Humphreys R, Fulda S (2012) RIP1 protein-dependent assembly of a cytosolic cell death complex is required for inhibitor of apoptosis (IAP) inhibitor-mediated sensitization to lexatumumab-induced apoptosis. J Biol Chem. 287:38767-38777.

Chen Q, Zhang X-N, Yu H-W, Wang Y, Tang H-R (2012) Changes of total anthocyanins and proanthocyanidins in the developing blackberry fruits. Int J ChemTech Res. 4:129-137.

Chew AL, Jessica JJA, Sasidharan S (2012) Antioxidant and antibacterial activity of different parts of *Leucas aspera*. Asian Pac J Trop Biomed. 2:176-180.

Christofferson DE, Li Y, Hitomi J, Zhou W, Upperman C, Zhu H, Gerber SA, Gygi S, Yuan J (2012) A novel role for RIP1 kinase in mediating TNFα production. Cell Death Dis. 3:e320.

Cordenunsi BR, Nascimento JRO, Genovese MI, Lajolo FM (2002) Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. J Agric Food Chem. 50:2581-2586.

Cragg GM, Newman DJ (2013) Natural products: a continuing source of novel drug leads. Biochim Biophys Acta. 1830:3670-3695.

Do JC, Son KH, Kang SS (1998) Studies on the constituents of the Roots of *Rubus parvifolius*. Korean J Pharmacogn. 19:170-173.

Fecker LF, Stockfleth E, Braun FK, Rodust PM, Schwarz C, Köhler A, Leverkus M, Eberle J (2010) Enhanced death ligand-induced apoptosis in cutaneous SCC cells by treatment with diclofenac/hyaluronic acid correlates with downregulation of c-FLIP. J Invest Dermatol. 130:2098-2109.

Ghosh D, Konishi T (2007) Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. Asia Pac J Clin Nutr. 16:200-208.

Gull J, Sultana B, Anwar F, Naseer R, Ashraf M, Ashrafuzzaman M (2012) Variation in antioxidant attributes at three ripening stages of guava (*Psidium guajava* L.) fruit from different geographical regions of Pakistan. Molecules. 17:3165-3180.

Gura T (1997) How TRAIL kills cancer cells, but not normal cells. Science. 277:768.

Hsieh P-C, Huang G-J, Ho Y-L, Lin Y-H, Huang S-S, Chiang Y-C, Tseng M-C, Chang Y-S (2010) Activities of antioxidants, α-glucosidase inhibitors and aldose reductase inhibitors of the aqueous extracts of four *Flemingia* species in Taiwan. Bot Stud. 51:293-302.

Hyun TK, Eom SH, Kim J-S (2014) Molecular docking studies for discovery of plant-derived α-glucosidase inhibitors. Plant Omics. 7:166-170.

Hyun TK, Kim M-O, Lee H, Kim Y, Kim E, Kim J-S (2013) Evaluation of anti-oxidant and anti-cancer properties of *Dendropanax morbifera* Leveille. Food Chem. 141:1947-1955.

Hyun TK, Lee S, Rim Y, Kumar R, Han X, Lee SY, Lee CH, Kim JY (2014) De-novo RNA sequencing and metabolite profiling to identify genes involved in anthocyanin biosynthesis in Korean black raspberry (*Rubus coreanus* Miquel). PLoS One. 9:e88292.

Irshad M, Zafaryab M, Singh M, Rizvi MMA (2012) Comparative analysis of the antioxidant activity of *Cassia fistula* extracts. Int J Med Chem. 2012: 157125.

Kim EJ, Lee YJ, Shin HK, Park JH (2005) Induction of apoptosis by the aqueous extract of *Rubus coreanum* in HT-29 human colon cancer cells. Nutrition. 21:1141-1148.

Kim J-S, Kwon Y-S, Chun W-J, Kim T-Y, Sun J, Yu C-Y, Kim M-J (2010) *Rhus verniciflua* stokes flavonoid extracts have anti-oxidant, anti-microbial and α-glucosidase inhibitory effect. Food Chem. 120: 539-543.

Kim HS, Park SJ, Hyun SH, Yang SO, Lee J, Auh JH, Kim JH, Cho SM, Marriott PJ, Choi HK (2011) Biochemical monitoring of black raspberry (*Rubus coreanus* Miquel) fruits according to maturation stage by 1H NMR using multiple solvent systems. Food Res Int. 44:1977-1987.

Kim TG, Kang SY, Jung KK, Kang JH, Lee E, Han HM, Kim SH (2001) Antiviral activities of extracts isolated from *Terminalis chebula* Retz., *Sanguisorba officinalis* L., *Rubus coreanus* Miq. and *Rheum palmatum* L. against hepatitis B virus. Phytother Res. 15:718-720.

Kumar S, Narwal S, Kumar V, Prakash O (2011) α-glucosidase inhibitors from plants: A natural approach to treat diabetes. Pharmacogn Rev. 5:19-29.

Lee J, Dossett M, Finn, CE (2012) Rubus fruit phenolic research: The good, the bad, and the confusing. Food Chem. 130:785-796.

Lim JW, Hwang HJ, Shin CS (2012) Polyphenol compounds and anti-inflammatory activities of Korean black raspberry (*Rubus coreanus* Miquel) wines produced from juice supplemented with pulp and seed. J Agric Food Chem. 60:5121-5127.

Notarbartolo M, Poma P, Perri D, Dusonchet L, Cervello M, D'Alessandro N (2005) Antitumor effects of curcumin, alone or in combination with cisplatin or doxorubicin, on human hepatic cancer cells. Analysis of their possible relationship to changes in NF-kB activation levels and in IAP gene expression. Cancer Lett. 224:53-65.

Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, Dixit VM (1997) The receptor for the cytotoxic ligand TRAIL. Science. 276:111-113.

Park IJ, Lee YK, Hwang JT, Kwon DY, Ha J, Park OJ (2009) Green tea catechin controls apoptosis in colon cancer cells by attenuation of $H_2O_2$-stimulated COX-2 expression via the AMPK signaling pathway at low-dose $H_2O_2$. Ann N Y Acad Sci. 1171: 538-544.

Park J-H, Jeong YD, Hassan MR, Kang CW, Ryu KS (2012) Effect of *Rubus coreanus* Miquel byproducts on performance and hormone secretion of crossbred chicks. Asian J Anim Vet Adv. 7:970-979.

Pedrielli P, Holkeri LM, Skibsted LH (2001) Antioxidant activity of (+)-catechin. Rate constant for hydrogen-atom transfer to peroxyl radicals. Eur Food Res Technol. 213:405-408.

Sakamaki K, Satou Y (2009) Caspases: evolutionary aspects of their functions in vertebrates. J Fish Biol. 74:727-753.

Seo YC, Choi WY, Kim JS, Yoon CS, Lim HW, Cho JS, Ahn JH, Lee HY (2011) Effect of ultra high pressure processing on immuno-modulatory activities of the fruits of *Rubus coreanus* Miquel. Innov Food Sci Emerg. 12:207-215.

Shin TY, Kim SH, Lee ES, Eom DO, Kim HM (2002) Action of *Rubus coreanus* extract on systemic and local anaphylaxis. Phytother Res. 16:508-513.

Surveswaran S, Cai Y-Z, Corke H, Sun M (2007) Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem. 102:938-953.

Sutherland BA, Rahman RM, Appleton I (2006) Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration. J Nutr Biochem. 17:291-306.

Taniguchi S, Kuroda K, Doi K, Inada K, Yoshikado N, Yoneda Y, Tanabe M, Shibata T, Yoshida T, Hatano T (2007) Evaluation of gambir quality based on quantitative analysis of polyphenolic components. Yakugaku Zasshi. 127:1291-1300.

Yoon I, Wee JH, Moon JH, Ahn TH, Park KH (2003) Isolation and identification of quercetin with antioxidative activity from the fruits of *Rubus coreanum* Miquel. Korean J Food Sci Technol. 35:499-502.

Yu Y, Deng Y, Lu BM, Liu YX, Li J, Bao JK (2014) Green tea catechins: a fresh flavor to anticancer therapy. Apoptosis. 19: 1-18.

Wang LS, Stoner GD (2008) Anthocyanins and their role in cancer prevention. Cancer Lett. 269:281-290.

Xu Y, Zhang Z, Li L, Joshi MK, Huang N, Niu J, Lu Y (2013) Catechins play key role in green tea extract-induced postprandial hypoglycemic potential in vitro. Eur Food Res Technol. 237:89-99.

Zhang J, Wang X, Yu O, Tang J, Gu X, Wan X, Fang C (2011) Metabolic profiling of strawberry (*Fragaria* x *ananassa* Duch) during fruit development and maturation. J Exp Bot 62:1103-1118.